SALAMANDRA **60**(4): 297–301
15 November 2024 | ISSN 0036–3375

SALAMANDRA
German Journal of Herpetology

Correspondence

Davian behaviour and functional egg fertilization in the Italian Stream Frog *Rana italica* (Anura: Ranidae)

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Manuscript received: 12 July 2024
Accepted: 7 October 2024 by Katharina Ruthsatz

Behavioural ecology is the branch of biology that studies behavioural interactions between individuals at both population and community level. Davian behaviour, also referred as necrophiliac behaviour or thanatophilia, involves the sexual interaction between a living male and a deceased conspecific, either it being a male or a female (Dickerman 1960, Pintanel et al. 2021). This kind of behaviour occurs with rarity in animal populations, and it is speculated that mostly involve young males that do not have access to living mating partners for hierarchical or social position (Meshaka 1996, Costa et al. 2010). Davian behaviour has been reported in all the major groups of tetrapods but is especially frequent in ectotherms such as amphibians and reptiles, which have explosive reproductive events (Wells 2007, Izzo et al. 2012). There are several reports of necrophiliac behaviour in anuran amphibians (frogs and toads), and Pintanel et al. (2021) offer a review of studies which report both intraspecific and interspecific cases of necrophilia in amphibians. According to Sindaco & Razzetti (2021) there are 41 species of amphibians in Italy, 23 of which belong to the Order Anura (four taxa are naturalized aliens). Among them, Davian behaviour has already been reported in four species that inhabit the Italian peninsula: *Bombina variegata* (Linnaeus, 1758) (Sinovas 2009); *Bufo bufo* (Linnaeus, 1758) (Eibl-Eibesfeldt 1950); *Bufo spinosus* (Daudin, 1803), both at intraspecific (Marco & Lizana 2002, Ayres 2010) and interspecific level (Ayres 2010); *Rana temporaria* (Linnaeus, 1758) (Mollov et al. 2010).

Terms like "functional necrophilia" have been previously used in describing thanatophiliac behaviours, like a male anuran that triggers the release of oocytes from the abdominal cavities of a deceased female fertilizing them (e.g., Izzo et al. 2012). "Functional egg fertilization", as used here, refers only to the successful fertilization of eggs under unconventional circumstances, and better captures the focus on the reproductive outcome rather than the potential behaviour leading to it.

The Italian Stream Frog (*Rana italica* Dubois, 1987) is an Italian endemic anuran species that is distributed throughout the Apennines and can be found from 15 to 1800 meters above sea level; it is active both during the day and night preferring fast-flowing and well oxygenated clear streams with rocky beds, but also creeks, small ponds or artificial troughs and, rarely, temporary water bodies (Guarino & Sindaco 2016, Fagotti et al. 2019, Di Toro & Marini 2024). Within the European brown frogs, *R. italica* is the species that is majorly connected to the aquatic environments (Picariello et al. 2007), and its presence in lentic waters of low elevation is very sporadic (Di Toro & Marini 2024). Environmental and microclimatic conditions play an important role in the behavioural ecology of the Italian Stream Frog that can be active throughout the year or also hibernate in colder months (Guarino & Sindaco 2016). Reproduction can occur from January to late spring, with elevation significantly affecting the mating period (Buono et al. 2014) and with clutches exceptionally reported in water at 4°C (Guarino et al. 1993). Hence, the reproductive events of this anuran are not particularly explosives, principally due to the permanent characteristic of its mating habitats, unlike other Ranidae for

which copulation and egg deposition are connected to access to temporary pools, e.g., *Rana dalmatina* (Fitzinger, 1839) (Bernini et al. 2007). The amplexus is axillary, and the female lays up to 600 eggs in up to five clutches that are anchored under stones or to the vegetation on the bottom, in pools or in sheltered stream bends, where water flows slowly (Guarino et al. 1993, Picariello et al. 2007, Di Toro & Marini 2024). After spawning, males tend to stay in the water longer than females. The duration of the embryonic development, from fecundation to hatching, ranges within 15 and 30 days, whereas the larval phase lasts two to three months and is strongly influenced by the water temperature (Guarino 1992, Picariello et al. 2007). Despite numerous studies on its cytogenetics, systematics, phytogeography, age structure and reproductive biology (Guarino & Picariello 2014), very little is known regarding the behavioural ecology of this anuran. Recently, a defensive, possibly eye-protective, behaviour of *R. italica*, expressed to avoid injuries during subjugation, ingestion, and regurgitation by predators has been reported for the first time (Guarino et al. 2022), but there were no reports of thanatophilia in this species. In this note a case of Davian behaviour in the Italian Stream Frog (*Rana italica*) from a small stream of Central Italy is reported.

A female Italian Stream Frog (*Rana italica*) was found dead in a creek on the 12th of March 2023 within the municipality of Montegabbione, Umbria Region, Central Italy (WGS84: 42.920, 12.073) at roughly 420 m a.s.l. The habitat where the animal was found, as well as some of its morphological features such as the typical colouration pattern of the gular region, blackish with a whitish medial line, allowed to taxonomically identify the individual as an Italian Stream Frog (Picariello et al. 2007). Moreover, this anuran is abundant along the surveyed creek, where no other brown frogs are present. The animal was lying on its back sunk in a shallow, 15–20 cm depth, fast-flowing stream in a relatively advanced state of decomposition (Fig. 1A). The individual was photographed without handling or removing the carcass from the original site.

The developmental stage of the embryos was retrospectively staged using documentative photographs according to the Gosner generalized staging table (Gosner 1960).

Historical data minimum and maximum air temperatures from the area registered were retrospectively obtained from the 3BMeteo Italian portal (www.3bmeteo.com/meteo/montegabbione/storico/ accessed on 22.05.2024) for both the day of the observation and from the 31 days before the observation (i.e., 10.02.–11.03.). Average and standard deviation of temperatures were calculated.

The individual had clearly visible signs of decomposition, as demonstrated by the autolytic stage of phalanxes, and putrefaction due to macroscopic fungal hyphae sur-

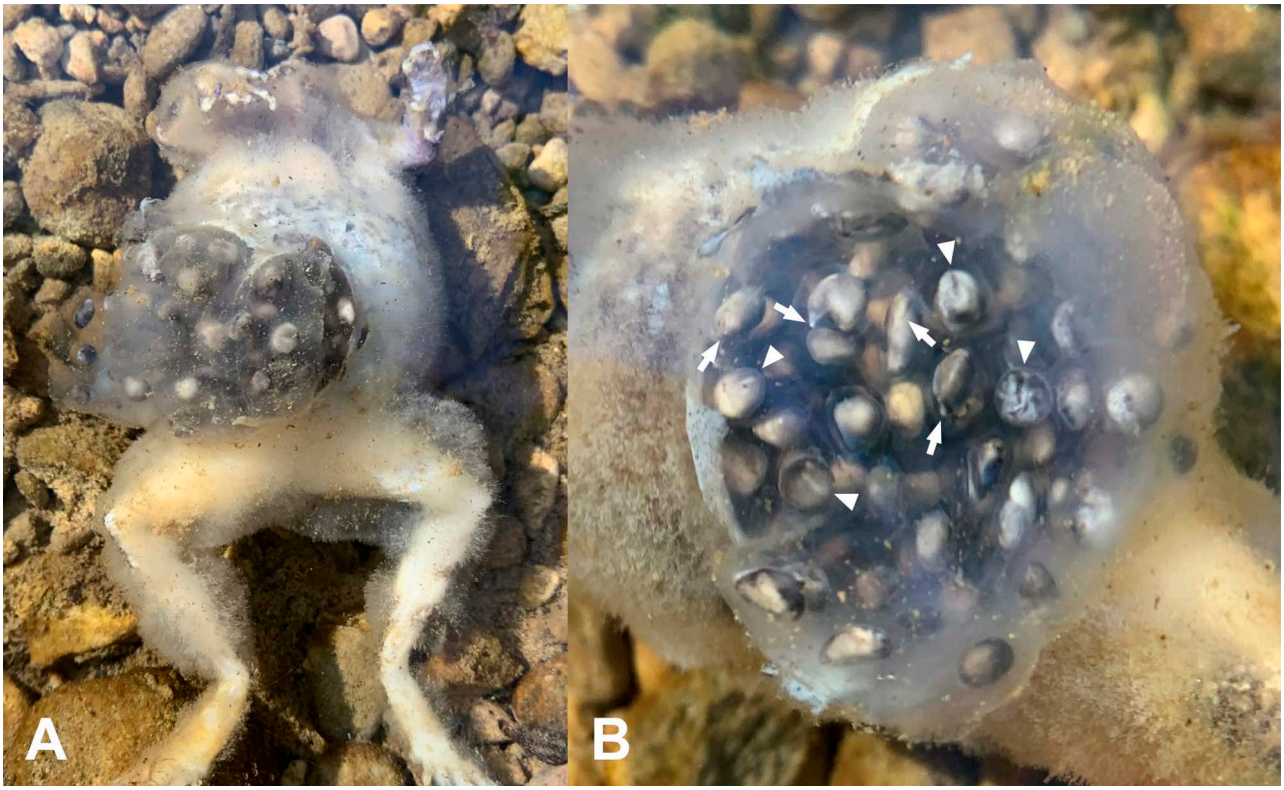

Figure 1. Photographs of the *Rana italica* carcass showing the partially fertilised egg mass protruding from the laceration of trunk wall. (A) Ventral view of the whole carcass; (B) close-up of the egg mass exhibiting different stages of embryonic development (Gosner stages 13–16: arrowheads; Gosner stage 17: arrows).

rounding the body (Fig. 1A). The frog presented an extended laceration at the level of the ventrocaudal wall of the trunk, in close proximity to the left hind leg. An egg cluster protruded from the breach, with both eggs facing the environment and eggs still inside the body cavity. Viable embryos in development, degenerating eggs and eggs that were not fertilized were present. The majority of the visible eggs were surrounded by their jelly coat layers. The embryos that were in development were motionless, and no twitching or spasmodic muscular responses were observed over the course of 1 hour. Different developmental Gosner stages (GS) were identified, mostly of which characterized embryos during neurulation. As a general pattern, the outermost embryos seemed to reach later stages compared to the deeper ones. The latest stage of embryonic development identified was stage 17, with immobile embryos characterized by a strongly arched shape and an evident tail bud (Figure 1B, arrows). Stages 13 to 16 are characterized by the process of transformation of the neural plate into neural tube: these stages were recognized by the presence of developing neural structures (Fig. 1B, arrowheads) in the innermost embryos.

The minimum and maximum air temperatures occurred during the day of the observation and the previous 31 days are reported in Figure 2. The average temperature on the 12 March and the previous 31 days was 7.1°C (± 5.1°C), of which the minimum and the maximum average temperatures were 2.9°C (± 2.9°C) and 11.2°C (± 3°C), respectively.

Most anurans, including the members of the Ranidae family, are characterized by external fertilization (Duellman & Trueb 1994). On the other hand, anuran eggs cannot be fertilizable prior to their transit in the pars convoluta of the oviduct, in which the vitelline membrane is matured to be penetrable to sperm (Elinson 1973, Katagiri 1974). During the passage in the oviduct, a jelly coat layer that contains molecular factors required for fertilization, is secreted and apposed around the eggs (Metz 1967). This implies that ovulated mature oocytes arrested in metaphase II, also called coelomic eggs, cannot be fertilized. Upon getting in contact with water the jelly coat tends to absorb water, expanding its volume and gradually losing essential water-soluble factors that are important for fecundation. Moreover, eggs must be quickly fertilized before the jelly coats absorb water and expand or before essential water-soluble components for fertilization disperse from the jelly (Hedrick & Nishihara 1991).

In the case here reported most of the eggs were embryonated and enclosed in jelly coats therefore meaning that they had to be passed through the oviduct of the female, in which they underwent the processes of maturation, and then liberated in the environment through the coelomic laceration. The presence of the jelly coat also suggests that fecundation occurred rapidly after the release of the eggs from the female body as, otherwise, the jelly coat enlarged by water absorption would have been an impenetrable barrier for spermatozoa. Furthermore, the presence of fungal hyphae all over the animal integument but not over the egg mass, that would otherwise have been putrefied and not fertilizable, suggests that a functional egg fertilization occurred right after the release of the egg from the laceration, most likely post-mortem.

Temperatures in Montegabbione during the 31 days prior to the observations (Fig. 2) are compatible with those suitable for the reproductive ecology of the Italian Stream Frog in other Italian regions (Guarino 1992). This makes the embryonic development of the reported case comparable to other reports. The latest embryonic stage observed was GS 17, represented by immobile embryos with a

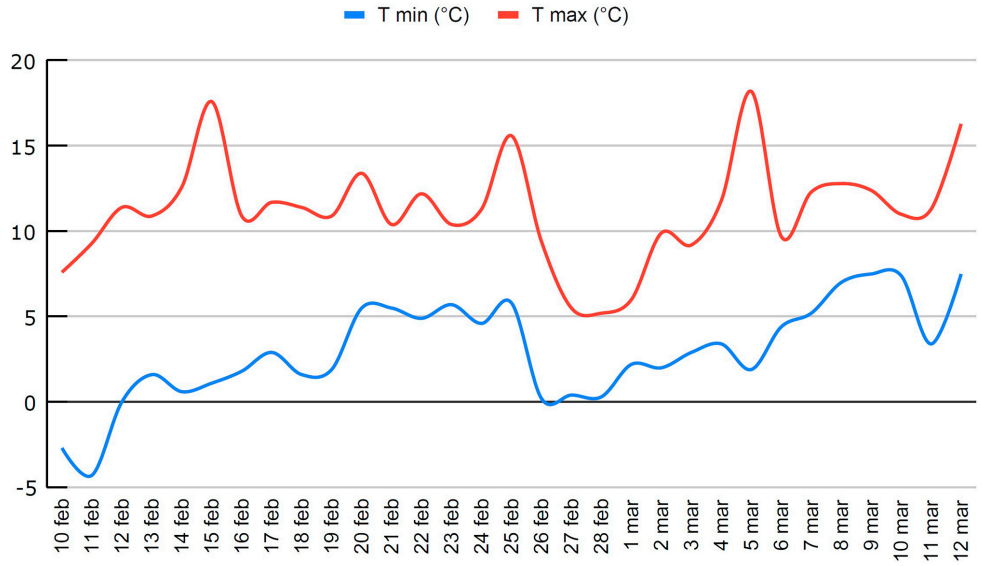

Figure 2. Variation of minimum (blue line) and maximum (red line) air temperature (°C) in Montegabbione (Terni) from February 10th to March 12th 2023.

strongly arched shape and a tail bud (Gosner 1960). This observation is strengthened by the fact that muscular responses in anuran embryos begin to be seen starting from GS 18, and were never noticed in the observed embryos. Since embryonic development in *R. italica* can last from 12 to 16 days (Guarino 1992), and since the more advanced stage embryos had not yet completed embryonic development, it can be hypothesized that fertilization occurred about ten days before the observation.

Unfortunately, estimating the accurate post-mortem interval (PMI) in amphibians and reptiles remains an obvious challenge due to their ectothermic characteristics (Cooper 2012). The PMI of the female frog from this report could not be estimated and compared with the fertilization time and there is no sufficient evidence to infer the causes of the laceration that could range from natural autolysis of tissue to predation or necrophagy. It is improbable that the female was killed and lacerated by a male during amplexus as the amplexus of *R. italica* is not very vigorous and there are no reports in literature of female of this species killed by males during mating. On the other hand, it is undoubtful that a male frog has functionally fertilized the eggs while they were still viable and their jelly coat was still penetrable, making of this the first report of Davian behaviour in *R. italica*. It has been suggested that necrophilia may serve a functional role in certain species, particularly those with highly skewed sex ratios favouring males (Pintanel et al. 2021). However, there is currently no published data regarding the sex ratio of *R. italica*, nor do we have observations on the sex ratio of the population present at our study site. Consequently, further research is needed to determine the contextual factors that may lead to this behaviour in *R. italica*. Nevertheless, the fact that, despite numerous field studies conducted in the past on *R. italica* (e.g., Guarino et al., 2014, Buono et al. 2014, Fagotti et al. 2019), our observation represents the first documented case of thanatophilia in this species suggests that such behaviour is quite rare (or perhaps occasional) among these anurans.

Funding

Costs of Open Access publication were covered by "Progetto di Ricerca di Base 2020" of Perugia University awarded to Livia Lucentini.

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